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ZOOLOGY

Strategies for Conservation of Endangered Amphibian and Reptile Species

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Abstract—Strategies for conservation of endangered amphibian and reptile species are discussed. One-fifth of all vertebrates belongs to the category of "endangered species," and amphibians are first on the list (41%). Every fifth reptile species is in danger of extinction, and insufficient information is characteristic of every other fifth. As has been demonstrated, efficient development of a network of nature conservation areas, cryopreservation, and methods for laboratory breeding and reintroduction play the key roles in adequate strategies for preservation of amphibians and reptiles.

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INTRODUCTION

The UN convention (1992) declared the ongoing value of biological diversity and its paramount importance for functioning of the systems maintaining both life in the biosphere and sustainable development of human society.

The scope of herpetologists' activity covers a wide range of issues associated with the integrated study and conservation of two vertebrate classes, amphibians and reptiles. The trends in population changes, threats of decline in biodiversity, and nature conservation strategies have both common patterns and serious differences determined by the history of their origin and specific physiological features (Orlov, 1982; Darevsky and Orlov, 1988; Goncharov et al., 1989; Serbinova et al., 1990a, 1990b).

The goal of this work was to discuss the strategies for preservation of the extant amphibian and reptile species using published data and our own data, as well as to clarify the need for efficient development of a network of nature conservation areas, cryoconservation, and methods for laboratory breeding and reintroduction.

INTERNATIONAL ACTIVITIES RELATED TO CONSERVATION OF AMPHIBIANS AND REPTILES

The founding of the International Union for Conservation of Nature and Natural Resources (IUCN) in 1948 marked a new stage in the research into threatened and endangered species of the global fauna and their preservation. IUCN is a powerful nongovernmental organization, uniting over 100 countries of the world. Manifold international projects are planned, coordinated, and implemented under the auspices of IUCN, and an ad hoc Species Survival Commission (SSC) was organized; this commission organizes groups of experts based on geographic and taxonomic principles. The results of their activities are reported on a weekly basis (http://www.iucn.org/themes/ssc/whats-new.htm). Despite dramatic changes in the environment, the nature conservation activities cannot be regarded as ineffective. The activities of this commission in recent decades have addressed analytical problems with the help of the expert community associated with assessment of risks and threats for vertebrate survival in general and its individual classes (Hoffmann et al., 2010; Böhm et al., 2013), as well as assessment of the possibilities of nature conservation efforts for reduction of biodiversity losses.

Vertebrates account for only 3% of all known animal species but play a significant role in ecosystems and are of paramount cultural and social value. Analysis of the data on the distribution, population dynamics, main threats, and conservation status of 25780 vertebrate species—mammals (5498 species), birds (10027 species), reptiles (a sample of 1500 species), amphibians (6638 species), and bony fishes (a sample of >1200 species)—has demonstrated that almost one-fifth of the extant species belongs to the "endangered" group, ranging from 13% for birds to 41% for amphibians (Hoffmann et al., 2010). The Red List Index (RLI), elaborated for assessment of changes in biodiversity based on the information about all nature conservation categories of the IUCN Red List of Endangered Species, was used in the analysis performed. This index determines the conservation status and is an important quantitative unit for measuring risks (Butchart et al., 2007).

Changes in RLI have been computed for birds, mammals, and amphibians (for the years of 1980 and 2004), since the data for trends are still unavailable for other vertebrate groups. The RLI values for amphibians, which are more endangered as compared with birds and mammals, changed by 3.4% from 1980 to 2004 (that is, by 0.14% annually). In particular, deterioration of the population state of amphibians has been observed for 662 amphibian species, which has changed the Red List categories towards extinction during the examined period. The IUCN categories for almost 40 amphibian species deteriorated by three or more units. At least nine amphibian species disappeared during the two decades following 1980 (including the golden toad *Incilius periglenes* from Costa Rica and two species of the unique Australian genus *Rheobatrachus*); in addition, 95 species, including 18 species of the Neotropical genus *Atelopus* (23% of the species in this genus) are regarded as extinct with a high probability. The threat has decreased and the level of conservation status has increased for only one amphibian species, the midwife toad *Alytes muletensis*, whereas these characteristics only worsened for 208 species.

The global crisis in the state of amphibians was recorded over 20 years ago and is still topical (Blaustein and Wake, 1995; Stuart et al., 2004). Among the major threats are destruction and pollution of habitats; climate changes; excess catching, eliminating these animals from wildlife; and excess use as food as well as a recently discovered severe infectious disease of amphibians (chytridiomycosis, caused by *Batrachochytrium dendrobatidis*), affecting the skin of adult individuals and the oral cavities of their larvae (Longcore et al., 1999). This disease causes epidemics in wildlife, wiping out whole amphibian populations.

Reptiles are even less studied from the standpoint of nature conservation as compared with amphibians. Only 35% of the species have been estimated in this respect (IUCN Red List of Threatened Species). The international expert team on reptiles (Böhm et al., 2013) attempted for the first time to make a global estimate for the risk of reptile extinction in the wild based on a representative sample of 1500 species (16% of the known species). Every fifth of the 10038 known reptile species is under the threat of extinction; another fifth species falls into the category of insufficiently studied (Böhm et al., 2013). The share of the endangered species is the highest for the inhabitants of freshwater aquatic bodies in tropic regions and ocean islands, in particular, in Central Africa and Southeast Asia, as well as for underground dwellers and burrowers. The planned efforts on species conservation should take into account the consequences of the prevalent threats for reptiles, namely, anthropogenic destruction and loss of habitats and their killing for food.

THE ROLE OF NATURE RESERVES AND CONSERVATION AREAS IN AMPHIBIAN AND REPTILE PRESERVATION

In the overwhelming majority of cases, the anthropogenic destruction of animal habitats is the main threat for existence of the extant amphibian species. Organization of the maximally developed network of nature conservation areas (NCAs), i.e., plots of land, water surface, and the airspace above, on all continents is of paramount importance for animal conservation in all countries of the world. In the Russian Federation, the status of these territories is specified in the law, according to which the natural complexes and objects of special conservation, scientific, cultural, esthetic, recreational, and sanitary significance are completely or partially withdrawn from economic use by the decisions of the corresponding executive bodies with specification of their protection status (The Federal Law, 1995, 2014). As of 2010, approximately 12000 (11937) NCAs of the federal, regional, and local levels have been specified in Russia (http://www.zapoved.ru/conservation/results). Of them, 266 NCAs are of the federal level, including 101 nature reserves, 41 national parks, and 69 state partial reserves as well as natural monuments, resorts, and several other types of NCAs. All federal-level NSAs cover approximately 580000 km^2 in 81 of the 84 federal entities, amounting to ~3.4% of the territory of the Russian Federation.

Note that NCAs are of special importance for amphibians and reptiles. Unlike many widely migrating mammals and birds, characteristic of most of the amphibian and reptile species are comparatively limited seasonal and diurnal migrations, reaching at best several kilometers. More or less large migrations are observed for only some large species of monitor lizards, snakes, crocodiles, and turtles, whereas the majority of amphibian species, small lizards, snakes, and turtles stay in a territory not exceeding several hundred and even tens of square meters during their entire lives. That is why their populations are to a considerably lesser degree affected by the insular ecology patterns associated with the conserved areas, in particular, the effect of insularization of the distribution range and imbalance between the colonization and extinction, as compared with the mammalian and avian populations. The issue of minimum sizes of conserved areas able to fulfill their protective function is also considerably less important for amphibians and reptiles for the very same reason. In many cases, it is sufficient for this purpose to conserve several hundred square meters of habitats specific for a particular species with the presence of adequate overwintering shelters. These specific features of amphibians and reptiles can allow for efficient conservation of the viable populations even in comparatively small plots of specialized mini nature reserves. The rapid expansion of conservation area networks, taking place worldwide, first and foremost, national parks and nature reserves, allows for an optimistic view on the future of populations of many amphibian and reptile species. An illustrative example is the Caucasian ecological region, which has been studied well from the standpoint of nature conservation (Tuniev et al., 2009), comprising a network of conservation areas of different levels, in particular, 51 nature reserves, 24 national parks, 162 partial reserves, numerous nature monuments, conservation landscapes, and so on.

BREEDING IN CAPTIVITY AS A METHOD OF GENE POOL CONSERVATION FOR THREATENED REPTILE AND AMPHIBIAN SPECIES

Breeding in captivity as a method of conservation of species diversity for threatened animal species has demonstrated its efficiency for many mammals and birds (Flint, 2004). Organization of the Conservation Breeding Specialist Group to coordinate selection of the flagship species for breeding projects demonstrates that IUCN recognized the role of laboratory breeding. The successful experience in creating cultures of amphibian species for scientific purposes, including the African clawed frog *Xenopus laevis*, the Mexican axolotl *Ambystoma mexicanum*, and the Spanish ribbed newt *Pleurodeles waltl* is widely known. The demand for these animals, having multiply increased in the world market, has led to intensified poaching but, on the other hand, enhanced elaboration of improved methods for their artificial breeding. According to the information regularly published in the *International Zoo Yearbook*, issued by The Zoological Society of London, hundreds of amphibian and reptile species, including the species from the IUCN red lists, are successfully bred and raised in zoos and specialized breeding centers worldwide. The issues of conservation and breeding, including laboratory breeding, are considered in specialized volumes of this yearbook (*International* ..., 1990, 2008). In particular, volume 42 presents the strategy of the Amphibian Group. This volume opens with a topical paper titled “Can Zoos and Aquariums Ensure the Survival of Amphibians in the 21st Century?” (Reid and Zippel, 2008).

Laboratories and zoos utilize the unique possibilities of observation, recording, and analysis of information about various aspects of the biology of secretive species. The most illustrative examples are the amazing discoveries of facultative parthenogenesis of the monitor lizards *Varanus panoptes* (Lenk et al., 2005), *V. ornatus* (Hennessy, 2010), and *V. komodoensis* (Watts et al., 2006). Specialized programs for breeding of these animals and their “herd books” have been developed and coordinated by the World Association of Zoos and Aquariums (WAZA), the European Association of Zoos and Aquariums (EAZA), and the Eurasian Regional Association of Zoos and Aquariums (EARAZA).

The number of bred endangered amphibian and reptile species is still relatively small; however, the laboratory breeding of amphibians and reptiles has considerably advanced over the past few decades. This success is based on the research into reproductive behavior and is associated with hormonal stimulation of the male and female sexual cycles, clarification of the optimal temperature modes for animal keeping, rational feeding of juvenile and adult animals, ultraviolet irradiation, computer-based monitoring during incubation, and other methods elaborated in specialized laboratories. Basically different approaches are practiced for amphibians and reptiles, providing for success in this field. Gonadotropic hormones are successfully used for breeding amphibians; these hormones stimulate reproductive behavior, amplexus, and spawning (Goncharov et al., 1989; Kouba et al., 2009, 2012). Most frequently, synthetic analogs of the luteinizing hormone (releasing hormone, LHRHa, and human chorionic gonadotropin, HCG) are used. These hormones have allowed the progeny of many amphibian species from the USSR Red List to be obtained and grown successfully (Goncharov et al., 1989; Serbinova et al., 1990a, 1990b). Some tropical amphibian species after several years of studies came to be model species for herpetoculture; the methods for laboratory breeding were successfully elaborated for these species as well as the construction of foam nests and larval development were described (Widenhues et al., 2011). Various exogenous impacts, first and foremost, light and temperature factors are applied much more widely in the breeding of reptiles.

Currently, state-of-the-art reproductive technologies, utilizing intravital obtaining of unfertilized spawn and sperm proper are actively being used for amphibians (Uteshev et al., 2003; Browne et al., 2011). Intravital obtaining of spawn and urinal sperm is also accompanied by hormonal stimulation of the oocyte ovulation and spermiation (Kouba et al., 2009, 2012). Methods for artificial spawn fertilization of tailless amphibians with both fresh and cryopreserved sperm have been developed (Browne and Figiel, 2010; Shishova et al., 2011; Uteshev et al., 2013). Of special interest are recent studies in which tailless amphibians were artificially fertilized in vitro internally (Mansour et al., 2011). Note that the state-of-the-art reproductive technologies of artificial spawn fertilization have not yet found a wide practical application in breeding amphibians in zoos and breeding centers. However, cryobanks of cryopreserved sperm will soon appear in many breeding facilities and use of artificial fertilization with such cryogenic sperm will become a common practice.

The first Russian publications on practical success in the breeding of tropical and temperate-latitude reptiles appeared in the late 1970s–early 1980s (Igolkina et al., 1977; Orlov, 1982). The principles for creating

Table 1. Data from the EARAZA reports for 2004–2012 on reproducing amphibian groups in zoological collections

Year	Number of		Order		
	countries	zoological institutions	Apoda	Caudata	Anura
2012	17	109	One family and one species	Two families and 11 species	Ten families and 41 species
2011	17	108	The same	Two families and eight species	11 families and 39 species
2010	17	109	"	One family and five species	Nine families and 32 specie
2009	17	109	"	The same	Ten families and 35 species
2008	16	104	"	One family and six species	Ten families and 34 species
2007	16	101	One family and two species	Two families and seven species	Nine families and 21 species
2006	16	99	The same	One family and five species	Five families and 19 species
2005	15	96	One family and one species	The same	Eight families and 17 species
2004	16	95	One family and two species	One family and four species	Eight families and 19 species

artificial populations and the concept of effective introduction of this method to the set of nature conservation measures appeared somewhat later along with organization of the priority nature conservation areas (Darevsky and Orlov, 1988). So far, zoos and breeding centers both around the world and in this country have accumulated a rich experience in this field (Kudryavtsev et al., 1991). Noticeable results have been obtained in breeding of known reptile species, such as the Komodo dragon and Indian monitors, in the London, Rotterdam, and Prague zoos (Velensky, 2007; Visser et al., 2009). Analysis of the EARAZA reports on reproducing amphibian and reptile groups, comprising the data of over 100 zoological institutions from 15–17 European and Asian countries gives representative results (Tables 1, 2). From 2004 to 2012, the species diversity of anuran and caudata amphibians has considerably increased resulting in higher representation of the anuran families. The same trend in reptiles is characteristic of turtles and lizards, whereas species diversity in the zoological collections of reproducing snakes has decreased.

The logical continuation for the activities on amphibian and reptile breeding in captivity is the reintroduction (or repatriation) of the bred animals into their natural habitats. Considering this aspect for amphibians and reptiles, it is evident that their release into wildlife is not accompanied with the specific difficulties characteristic of reintroduced mammals and birds bred in captivity. First and foremost, this is associated with the absence of the so-called altricial period, characteristic of altricial animals, as well as the habituation to ambience and people, which is typical of many mammals and birds bred in captivity. Thus, repatriation or reintroduction programs for amphibians and reptiles as a rule encounter no serious complications. In this case, there is no need to use the so-called rewilding, which may have specific features when dealing with different animal groups. Not only young frogs, salamanders, turtles, lizards, and snakes, but also the adult individuals that spent a long time in captivity rapidly settle in the natural ambience and, having successfully reproduced, form stable populations with time. In particular, the true lizards of the genera *Lacerta* and *Podarcis* introduced from Europe successfully settled in some regions of the United States (Gubanyi, 2000; Deichsel and Gist, 2001; Kalyabina-Hauf and Deichsel, 2002); a brown anole that was accidentally brought to the southern islands of Japan widely spread there (Norval et al., 2002); some exotic lizards, snakes, and turtles originating from terrariums live and successfully reproduce in southern Europe; and skinks and geckos shipped to ocean islands widely spread there. It is also confirmed that the Turkestan thin-toed gecko (*Tenuidactylus fedtschenkoi* (Strauch, 1887)) lives in the region of Odessa, Ukraine (Duz' et al., 2012).

Not infrequently, the introduced amphibians and reptiles start competing with aboriginal species, become dangerous predators, and even drive them out. The best textbook examples are the planned introduction of the giant toad (*Bufo marinus*) and the acciden-

Table 2. Data from the EARAZA reports for 2004–2012 on reproducing reptile groups in zoological collections

Year	Number of		Order			
	countries	zoological institutions	Chelonia	Crocodilia	Sauria	Serpentes
2012	17	109	Five families and 17 species	Two families and two species	11 families and 63 species	Four families and 82 species
2011	17	108	Three families and 14 species	The same	11 families and 54 species	Four families and 75 species
2010	17	109	Four families and 18 species	Two families and three species	12 families and 45 species	Four families and 81 species
2009	17	109	Four families and 12 species	One family and one species	Ten families and 41 species	Four families and 92 species
2008	16	104	Five families and 16 species	Two families and two species	Ten families and 38 species	Four families and 107 species
2007	16	101	Five families and 15 species	One famil and one species	Ten families and 48 species	Four families and 102 species
2006	16	99	Three families and 12 species	The same	Nine families and 48 species	Four families and 103 species
2005	15	96	Three families and 12 species	Two families and two species	Eight families and 44 species	Four families and 98 species
2004	16	95	Two families and six species	The same	Seven families and 34 species	Four families and 138 species

tal spread of the brown treesnake (*Boiga irregularis*) beyond their distribution ranges. The giant toad was brought from the south of the North, Central, and South Americas for insect control to the Antilles, Hawaii, Fiji, Philippines, Japan (Ryukyu Islands), Taiwan, New Guinea, Pacific islands, and Australia (Urban et al., 2007), where its abundance now has become threatening to the aboriginal fauna. The brown treesnake was accidentally brought to Guam and has become a real disaster for the local bird species, insectivorous mammals, and lizards (Colvin et al., 2005). These processes (introduction and acclimation) in their consequences represent a potential source of biological pollution, which is an extremely dangerous and yet very widespread phenomenon (Flint, 2004).

The first success in reintroduction of domestic herpetofauna dates back to the USSR (1985–1987), namely, the activities of the working group on development of the scientific methods for breeding of amphibians and reptiles, organized by Flint (2004). Over 2000 individuals of the Syrian spadefoot (*Pelobates syriacus*) bred at the Moscow Zoo were released in Armenia. The subsequent observations demonstrated that part of the released toads settled successfully. A new stable group of the Syrian spadefoot was formed near the Azat artificial reservoir and is successfully spawning every spring (*The Red* ..., 2010).

Breeding for reintroduction should be always controlled by experts to provide for the genetic purity of released animals (*Guidelines*..., 2013). In the case of crosses in captivity, hybrids between species and especially between subspecies may readily appear, and it is inadmissible to pollute wildlife with such hybrids. The reintroduction of the species bred in captivity is desirable only provided that their habitats are retained in nature. This is also true for rather frequent cases when insular habitats are considerably "polluted" with introduced mammals, in particular, rats, which eat all the eggs and juveniles of lizards, snakes, and turtles. For example, the release of the giant tortoise juveniles in some of the Galapagos Islands, where only adult individuals of these reptiles can survive, is rather senseless.

How can zoos and breeding centers enhance the conservation of amphibian and reptile diversity? The most important tasks for zoos are breeding of threatened animal species and educational and informational activities. Zoos can and should engender a careful attitude towards nature and the environments in which animals and plants live, including unnoticeable animals, such as amphibians and reptiles. Properly made displays elevate the quality of knowledge about these animals as well as an understanding of their necessary role in wildlife as a component of biota.

By studying reproductive biology and creating artificial populations, breeding centers during periods of global crisis may act as modern "arks" to preserve wild animals in danger of extinction as well as to accumulate and maintain the reserve populations of endangered species (Browne et al., 2011). In an ideal situation, the combination of field studies in wildlife populations and elaboration of laboratory breeding methods by the same research team is optimal. Such efficient examples are known in the practice of the Prague and Cologne zoos.

Genetic cryobanks, which collect and provide long-term storage of reproductive and somatic cells of the endangered species, may and should be a harmonious component of the breeding centers and nurseries. On the one hand, the intravital material sampled from a large number of genetically valuable parents may be used actively for maintaining zoocultures of endangered species. Active use of diverse genetic material preserved in cryobanks will allow the consequences of inbreeding to be avoided. On the other hand, genetic cryobanks play the role of a backup collection of viable genetic biomaterial for the case of natural or technogenic catastrophes possibly wiping out all living individuals of an endangered species. In such cases, the genetic material preserved in a frozen but viable form in cryobanks may be the last hope for recovery of exterminated species.

Such genetic cryobanks are already organized in both Russia (an experimental genetic cryobank with the Institute of Cell Biophysics, Russian Academy of Sciences; Gakhova et al., 2006) and abroad (National Amphibian Genome Bank with the Memphis Zoo, United States; Hassapakis, 2014; http://memphiszoo.org/amphibiangenomebank). The genetic cryobanks organized in the framework of this strategy will not only enhance adequate breeding of amphibians in captivity with retention of the necessary interspecific genetic diversity, but also guarantee conservation of the genetic material of the extant herpetofauna.

Thus, it is currently necessary to develop integrated nature conservation measures paying serious attention to preservation of areas, laboratory breeding of amphibians and reptiles, and replenishment of their natural population pool by reintroduction and repatriation.

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